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14. ABSTRACT : Bone loss that results from age-related and post-menopausal osteoporosis, and the resulting increase in bone fractures, is an important health problem in the United States and worldwide. The most widely prescribed therapies for osteoporosis include bisphosphonates such as, Fosamax, Actonel, and Boniva, parathyroid hormone, known as Forteo, and calcitonin, known as Miacalcin. However, side effects associated with these compounds have been reported. Bisposphonates, which halt bone destruction but do not affect bone formation, are associated with gastrointestinal problems when taken orally and may cause osteonecrosis in cancer patients and bone pain in other patients when administered intravenously. Forteo does actually increase bone formation but must be injected subcutaneously daily and if given for extended periods can increase bone resorption. Finally, Miacalcin has only a moderate affect on bone. Therefore, there is a need for safer and more efficacious osteoporoses therapies especially those that stimulate new bone formation. New bone formation results from adult mesenchymal stem cells developing into bone forming osteoblastic cells. However, recent data suggest that the ability of adult mesenchymal stem cells to develop into osteoblastic cells decreases as we age. Indeed, this may strongly contribute to osteoporosis. This suggests that using mesenchymal stem cells, which are derived from the individual that has osteoporosis, to treat osteoporosis may be a promising therapeutic approach. Unfortunately, the availability of mesenchymal stem cells is limited, especially in aged individuals. Therefore, we are proposing to derive mesenchymal stem cells form induced pluripotent cells. Induced pluripotent cells are cells taken from adult tissue, for instance fibroblasts from skin, which can be reprogrammed to develop into any cell in the body including mesenchymal stem cells. These cells could then be used to treat osteoporosis, an approach that has not been taken yet. We propose to optimize the conditions, specifically the surface on which cells are cultured in vitro, under which mesenchymal stem cells are derived from induced pluripotent cells. The concept of exploiting changes in the surface on which cells grow to optimize induced pluripotent stem cell development into mesenchymal stem cells is highly innovative. We will then examine whether these induced pluripotent cell derived mesenchymal stem cells are better in stimulating bone formation, relative to mesenchymal stem cells not optimized, in a mouse model of osteoporosis. This approach has never been examined and successful completion of the specific aims would suggest a novel and highly innovative therapeutic approach to age-related osteoporosis.

15. SUBJECT TERMS
Induced pluripotent stem cells, osteoporosis, nanotopography

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Final Report for Award Number W81XWH-11-1-0405

Induced pluripotent stem cell derived mesenchymal stem cells for attenuating age-related bone loss

Covering July 1, 2011 through June 30, 2012
and
December 12, 2012 through June 30, 2013

Principal Investigator: Henry J. Donahue, Ph.D.

Table of Contents

Introduction

Osteoporosis, both age-related and post-menopausal, is a huge health problem in the United States and indeed worldwide. Despite extensive research there remain few therapeutic approaches, with the exception of parathyroid hormone, that actually increase bone formation in osteoporotic patients. There are several limitations to the use of parathyroid hormone suggesting the need for continued research into anabolic therapies for osteoporosis.[1] Mesenchymal stem cell (MSC) differentiation towards the bone forming osteoblastic lineage decreases as a function of age and may contribute to age-related bone loss.[2] Therefore, MSC therapy may be beneficial in treating age-related bone loss. However, MSC availability decreases with age.[2] To overcome the problem of age-related reduced availability of MSC we propose to examine the bone anabolic potential of induced pluripotent stem cell (iPS) derived MSC in age-related bone loss. Unfortunately deriving MSC from iPS can require extended in vitro culture, which decreases the differentiation potential of MSC.[3] Since biomaterial surface characteristics, including stiffness and topography, can control MSC differentiation in vitro, including toward the osteoblastic lineage,[4,5] the goal of this project is to identify biomaterial surface characteristics that enhance differentiation of iPS toward MSC and MSC toward osteoblastic cells. Our hypothesis is that culturing iPS on nanotopographic surfaces results in enrichment of a population of cells exhibiting MSC characteristics. Continued culture of these iPS derived MSC on nanotopographies results in increased osteoblastic differentiation and increased potential to induce bone formation in senescent accelerated mice (SAMP6), a murine model of age-induced bone loss. Successful completion of the specific aims would suggest a novel and highly innovative therapeutic approach to age-related osteoporosis.

Body

On July 27, 2012 we requested a 6 month extension without funding for this project. This extension was approved December 12, 2012. Therefore, this final report represents progress from July 1, 2011 through June 30, 2012 and from December 12, 2012 through June 30, 2013.

During this 1.5 year project we made considerable progress towards the completion of tasks 1 and 2. However, we were unable to get to a point where we actually injected iPSC to osteopenic mice (task 3). This is due to the fact that the time it took to develop iPS was longer than initially anticipated. However, the progress we made in establishing iPS[6] and in demonstrating that polymer nanotopographies enhanced hMSC osteoblastic differentiation[7] warrant continued examination of the potential of polymer nanotopographies to enhance iPS osteoblastic differentiation. The following outlines our results as regards each task.

Task 1. Examine the hypothesis that specific nanoscale topographies will increase differentiation of iPS toward the MSC lineage. We will examine expression of MSC markers on iPS cultured on specific nanoscale topographies.

We were able to demonstrate that we could generate iPS that differentiated into mesenchymal stem cells. This is a critical first step in examining whether polymer nanotopographies affect iPS differentiation towards mesenchymal stem cells. Differentiation of the cells derived from iPSC using different treatments, were assessed for differentiation into osteoblast in vitro. Cells were cultured in vitro in a medium supplemented with osteogenic supplements. At 21 days following cultivation, cells were assessed for mineral deposition. Results indicated that cells derived by different treatments differentiated into osteoblasts and deposited bone in vitro but with varying degrees. TGF-b1 treated cells showed most mineral deposition. Interestingly, BMP-2 treated cells exhibited a higher potential to differentiate toward osteoblasts. Differentiation of the cells toward osteogenic and adipogenic lineage are shown in figure 1. Quantification of mineral deposition by the iPS cells was also evident. We are now in an excellent position to examine the effect of polymer nanotopographies on iPS differentiation to MSC.

Task 2. Examine the hypothesis that specific nanoscale topographies increase the differentiation of MSC identified in aim 1 toward the osteoblastic lineage. We will examine expression of osteoblastic phenotypic markers on iPS derived MSC cultured on nanotopographic biofilms.

While we have not yet examined the effect of polymer nanotopographies on iPS differentiation to MSC, we have demonstrated that polymer nanotopographies enhance hMSC differentiation towards the osteoblastic

lineage. We found that culture on specific scale (10-20 nm high) polymer nanoislands accelerates hMSC lineage commitment toward osteogenesis, as seen by increased alkaline phosphatase (AP) activity and mineralization (Fig. 2;[7]). We further observed that the accelerated differentiation of hMSCs on specific nanotopographies could be detected in the form of decreased stem cell surface markers (Fig. 3;[7]). That hMSCs lose stem cell surface markers (SSEA-4, CD73, CD105) on 11 nm high nanoislands indicates that these cells are no longer stem cells (or already committed to a specific fate, in this case osteogenesis). These data suggest that specific nanoscale topography potentiates hMSC differentiation toward the osteoblastic lineage and therefore has the potential to induce iPSC differentiation toward MSC. These results have been published in the Journal of Orthopaedic Research.

Task 3. Examine the hypothesis that MSC, derived from iPS, cultured on nanotopographies enhance bone formation, in a murine model of aged-induced bone loss, to a greater degree than MSC derived from iPS cultured on flat surfaces.

We have been successful in generating iPS cells. However, we have found that culturing iPS cells on nanotopographies to be more challenging than originally envisioned. Therefore we requested and were granted additional time but no additional funds to attempt to complete a revised statement of work. The revised statement of work included continuing work on Task 1 and 2 as written above but not Task 3.

To address the challenges we were facing we examined several different culture conditions (Fig. 4) that included varying the number of days iPS cells were cultured in suspension; growth on nanotopography coated coverslips with and without gelatin; and culturing on standard tissue culture plates, with and without gelatin coating, prior to culture on nanotopography coated covers with and without gelatin coating. For all the conditions shown in figure 4 iPS cells were cultured on flat, 11nm, 25nm or 45nm nanotopographies. Under all conditions the majority of the cells died. We were unable to identify a culture condition that resulted in a sufficient number of cells to complete FACS analyses. However, one condition (green square in Fig. 4) did seem to result in a higher number of cells than the other conditions. Photo micrographs of iPS cells cultures under the most favorable condition we could identify are shown in figure 5. It is unclear why culture on nanotopographies under various conditions resulted in iPS cell death, especially considering that that MSC cell thrive on nanotopographies. Clearly iPS cells respond to surface topography in a fundamentally different manner than do MSC. Future studies examining the mechanism underlying this difference may provide insight to the biophysical regulation of stem cells in general.

Key Research Accomplishments

- Demonstrated the ability to generate iPSC that differentiate into MSC that form bone in vivo.
- Demonstrated that polymer nanotopographies enhance osteoblastic differentiation of hMSC and therefore have the potential to do so for iPS.
- Demonstrated a fundamental difference in the manner in which iPS cells and MSC respond to surface topography

Reportable Outcomes

Lim, J.Y., Loiselle, A.E., Lee,J.S., Zhang, Y., Salvi, J. D., and **Donahue, H.J.** (2011) Optimizing the osteogenic potential of adult stem cells for skeletal regeneration. J. Orthopaedic Research, 29(11):1627-33.

Li, Feng Niyibizi, Christopher. Induction of induced Pluripotent Stem cells (iPSC) to Multotent Mesenchymal Stem Cells and in vivo Bone Formation. 58th Annual Meeting of the Orthopeadic Research Society, San Francisco CA, Feb 2012.

Conclusion

Our results suggest that we can indeed induce pluripotent stem cells to differentiate into bone forming osteoblastic cells. Additionally, surface nanotopography affects hMSC to differentiate to osteoblastic cells. However, there is a fundamental difference in the manner in which iPS cells and MSC respond to surface topography

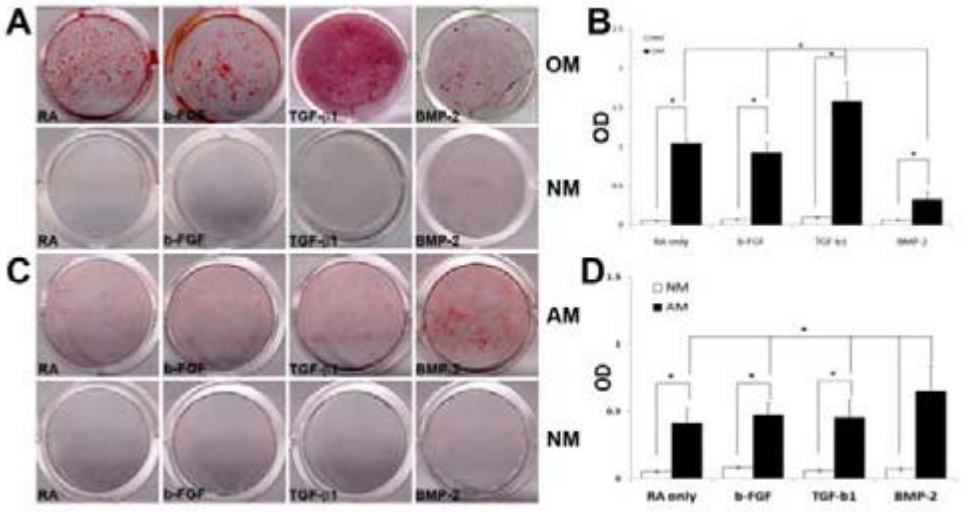

Figure 1.. Bone formation in vivo by iPSC TGF-derived MSCs with or without pre-BMP-2 treatment. A) pre-BMP-2treatment; B) No pre BMP-2 treatment. Pre-BMP-2 treatment enhances bone formation.

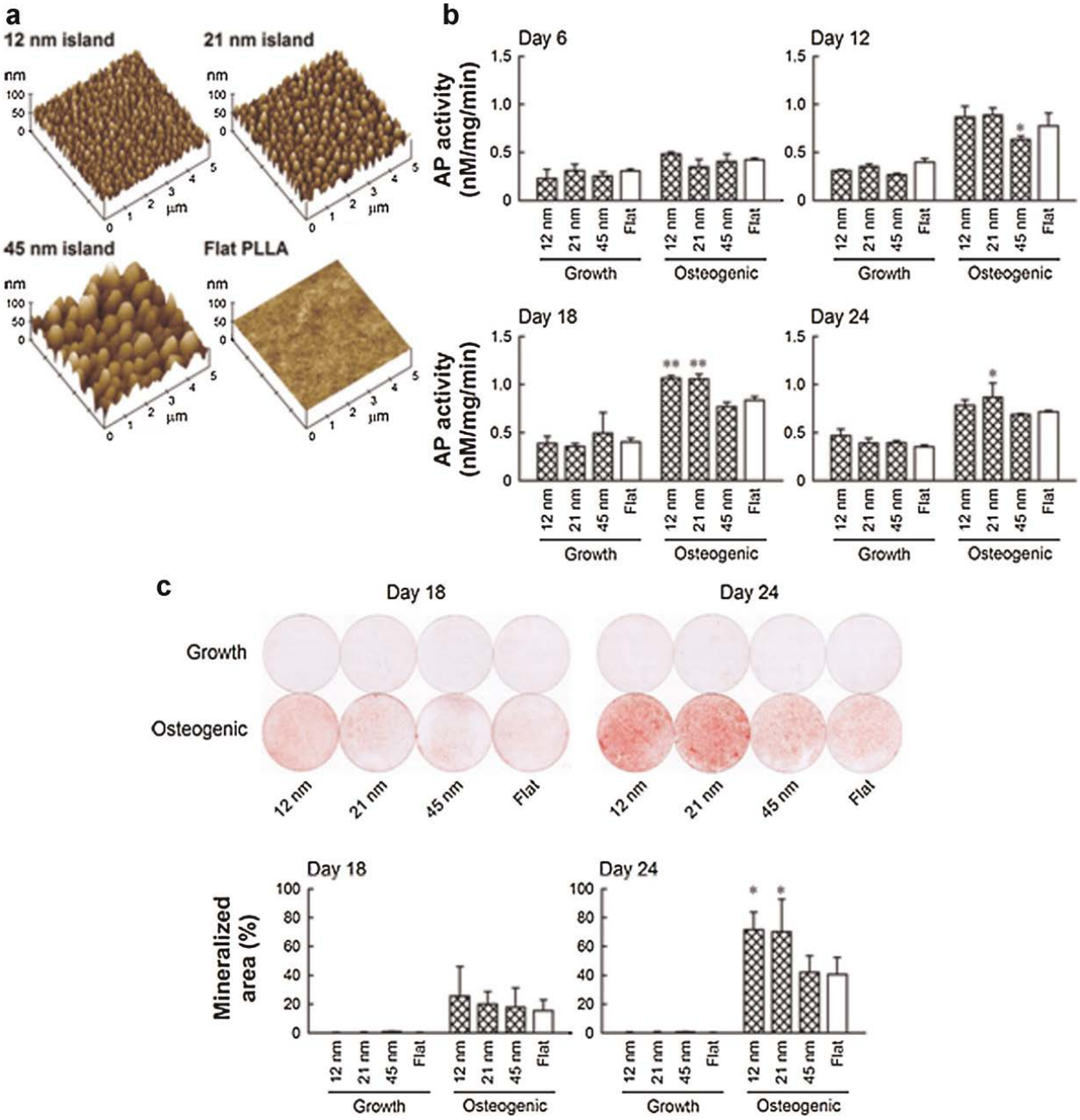

Figure 2. hMSC differentiation toward the osteoblastic lineage is enhanced when cells are cultured on specific nanoscale topographies. (a) Nanoisland topographies with varying island heights (12, 21, and 45 nm) produced by PLLA/PS (70/30 w/w) demixing. AFM height images of nanoislands and flat control surfaces are shown. (b) hMSCs were cultured on test surfaces in growth or osteogenic differentiation media and AP activity quantified (n=4). (c) Mineralized area. (n= 6).$p < 0.05$,$p < 0.01$ compared with flat PLLA; ANOVA with post hoc test.

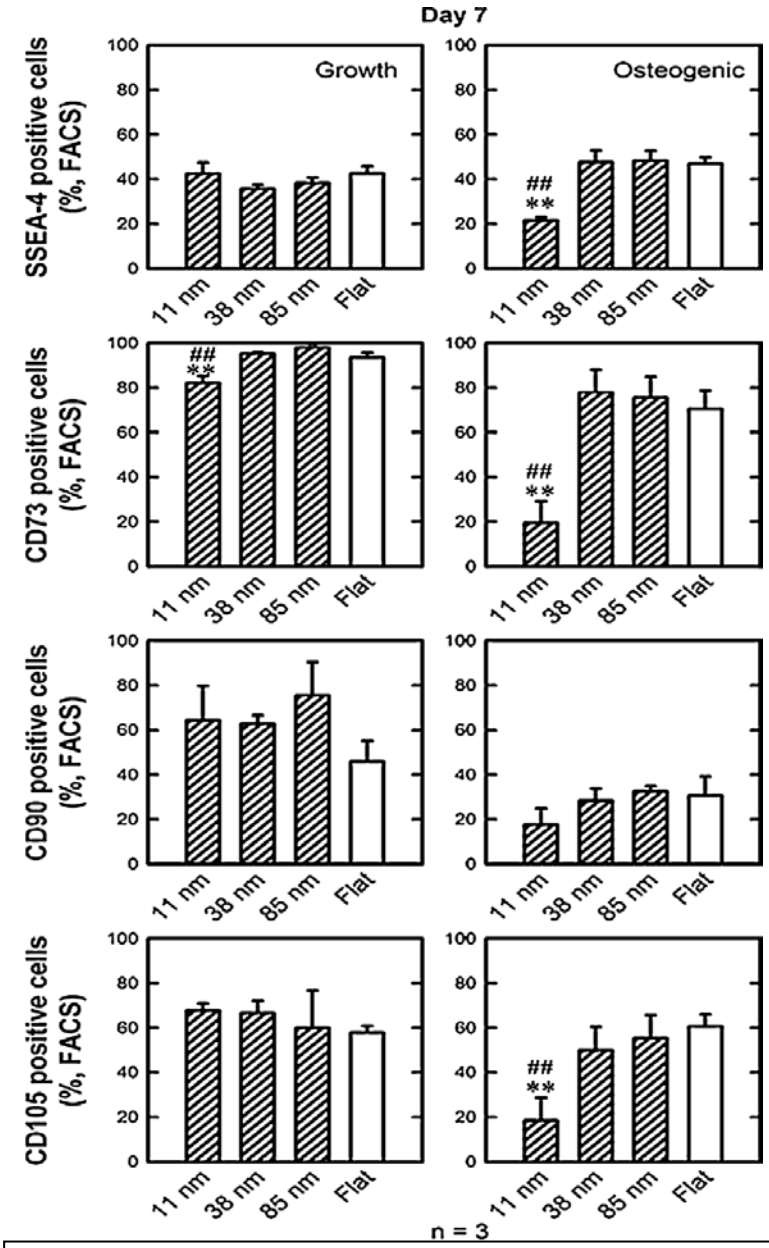

Figure 3. Stem cell surface marker expression in hMSCs on nanotopographies and flat control. Human MSCs were cultured on PS/PBrS films with growth or osteogenic media for 7 days, harvested, and tagged with antibodies for SSEA-4, CD73, CD90, and CD105. Flow cytometry was then utilized to determine the percent of the cell population with a positive stem cell surface marker. ##$p < 0.01$ compared among nanoislands; **$p < 0.01$ compared with flat control. ANOVA with post hoc test.

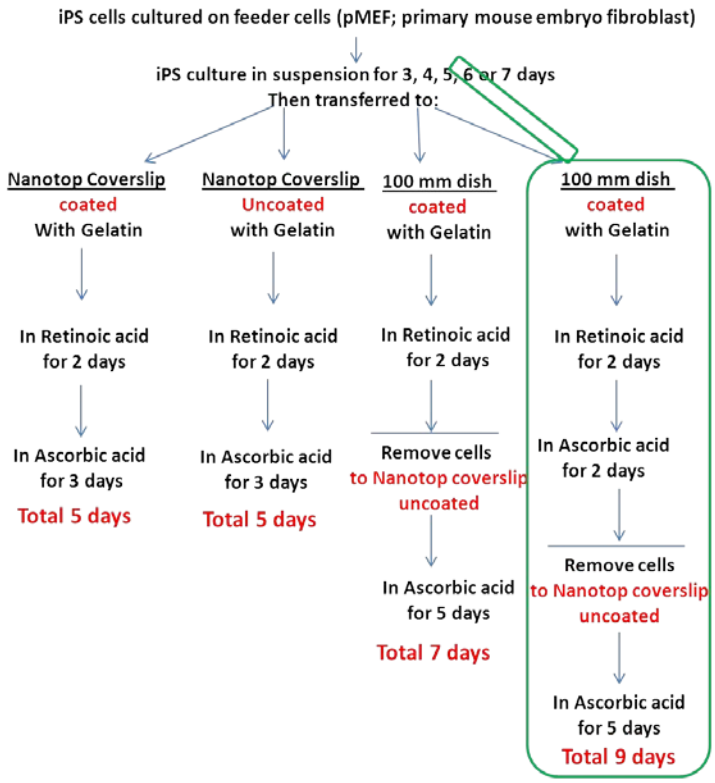

Figure 4. iPS cells were cultured on feeder cells for 2 days. The iPS cells were released from the feeder cells and culture in suspension for 3, 4, 5, 6 or 7 days. iPS cells were then cultured under the 4 conditions outlined above. The conditions outlined in green produced the most viable cells.

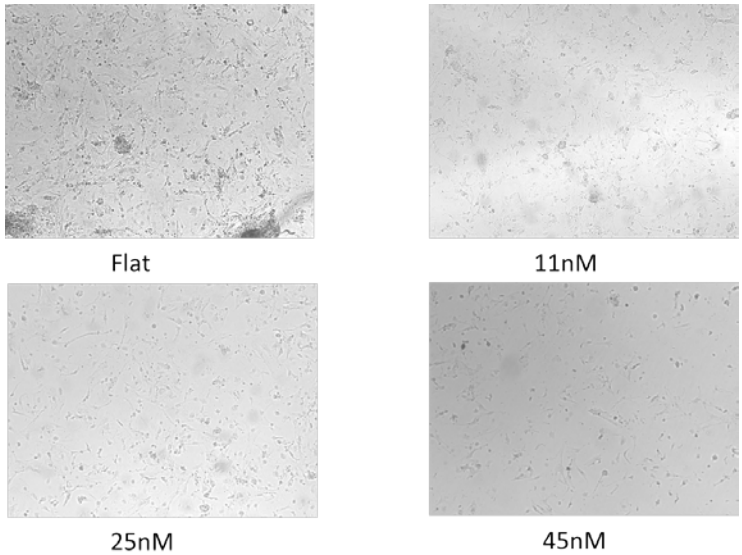

Figure 5. Photomicrographs of cell cultured on nanotopographies under the conditions outline in Figure 4.

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